

Morphological Variation in *Quinchamalium* (Schoepfiaceae) is Associated with Climatic Patterns along its Andean Distribution

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Abstract—*Quinchamalium* (Schoepfiaceae) is a root hemiparasite with a broad geographic range throughout the Andes. Regional studies have used various vegetative and floral traits to describe and identify species, but there has been no detailed analysis of the continuum of morphological variation across the entire geographic range of this genus. Currently 21 species names are being used in the genus but their taxonomic distinctiveness is unclear. The aim of this study was to use multivariate analyses to identify patterns of morphological variation, assess the existence of morpho-species, and correlate variation with climatic and geographic factors. Two putative species were initially circumscribed based on corolla length and this hypothesis was tested using principal component and discriminant analyses of 17 vegetative and floral characters obtained from 117 herbarium specimens. No statistically significant support was obtained through multivariate analyses for the existence of the two morpho-species, thus, only one species is recognized, a widespread and variable *Q. chilense*. Patterns of co-variation between several morphological traits and climate were identified. Taller plants with larger flowers were associated with sites with higher precipitation, and narrower leaves with higher temperatures. The presence of thrum flowers (floral morphs with relatively short styles) was correlated with higher latitudes and lower temperatures. Nevertheless, we have not determined whether these variations are genetically fixed ecotypes or are a consequence of phenotypic plasticity.

Keywords—Bioclimatic variables, ecotypes, geography, heterostyly, morphological continuum, morpho-species, phenotypic plasticity.

Identifying and delimiting species has always been a complex endeavor in systematics. The question of what defines a species is contentious, as evidenced by the existence of a large number of different species concepts (de Queiroz 2007). In systematics, being able to efficiently and accurately delimit species is basic because taxonomy is the fundamental study unit of different fields such as biogeography, ecology, and conservation. However, one of the major problems linked to species delimitation has been to distinguish between species concepts and species criteria (Wiens and Servedio 2000). One of the practical yet conservative strategies taken by many researchers has been to apply evidence from diverse sources to support the recognition of species. These include fixed or non-overlapping differences in morphological, behavioral, or ecological characters, molecular divergence, or geographic isolation. In this study, we recognize non-overlapping patterns of morphological variation as the primary criterion for inferring species boundaries, which is based on the idea that morphological discontinuities suggest that some evolutionary force may be preventing two distinct lineages from homogenizing (Stuessy 1990; Wiens and Servedio 2000). Morpho-species (Cronquist 1978) or pheno-species (Sokal and Sneath 1963) are those concepts that follow this basic criterion that has been applied in most of the morphological systematic studies (e.g. Lehnebach 2011; Lopez Laphitz et al. 2011; Nagahama et al. 2014). As posed by Wheeler and Meier (2000), even though a species definition may or may not make reference to characters (morphological or not), all concepts use character data to infer species boundaries, and most of those data are morphological. In this sense, we use the morpho-species criterion in an empirical example focusing on *Quinchamalium* Molina, a morphologically variable, geographically extended, and taxonomically understudied genus from southern South America.

Quinchamalium is a small genus of yellow-flowered hemiparasitic perennial herbs (Fig. 1) distributed throughout the Andes in open habitats across a wide elevational range (0–3,800 m a.s.l.). The genus was established by Molina in

1782 based on a specimen from Chile, and since then, 33 species names have been published within it. At present, *Quinchamalium* is thought to include approximately 21 species (Brako and Zarucchi 1993; Zuloaga et al. 2009; Jørgensen et al. 2014; Appendix 1), but the limits between these species are in many cases obscure (Dawson 1944). The genus is distributed from northern Peru to southern Chile, Argentina and Bolivia, covering a wide geographic area (Appendix 1). Despite this, the 21 accepted South American species mentioned before have not yet been treated in one single taxonomic study that includes them all. Consequently, no diagnostic key is available where the total number of recognized species of *Quinchamalium* are comparable. In the past, taxonomic treatments (Presl 1849; Philippi 1857; Miers 1880) included quantitative morphological traits related to flower and leaf morphology. More recently, Dawson (1944) and Navas (1976) developed regional taxonomic keys including some other quantitative characters. However, none of these taxonomic studies performed numerical analyses based on these quantitative traits when identifying key characters. In addition, there are recent examples in *Quinchamalium* where taxonomic distinctions were focused only on specimens from limited areas (Navas 1976: Cuenca de Santiago; Dawson 1984: Patagonia; Ulibarri 1994: San Juan, Argentina). Such studies may unnecessarily split a taxon into multiple taxa. On the other hand, a preliminary morphological examination of *Quinchamalium* specimens revealed considerable continuous variation in the characters used to differentiate these purported species.

Geographic variation in plant morphology can be a function of genetic changes (e.g. Quiroga et al. 2002; Premoli et al. 2007) or of phenotypic plasticity (e.g. Sultan 2000) in response to local environmental conditions. For this reason, the study and interpretation of patterns of geographic variation in morphological characters can potentially solve taxonomic problems as well as help to infer environmental effects. Moreover, the study of variation within widespread species at large spatial scales is the first step in determining

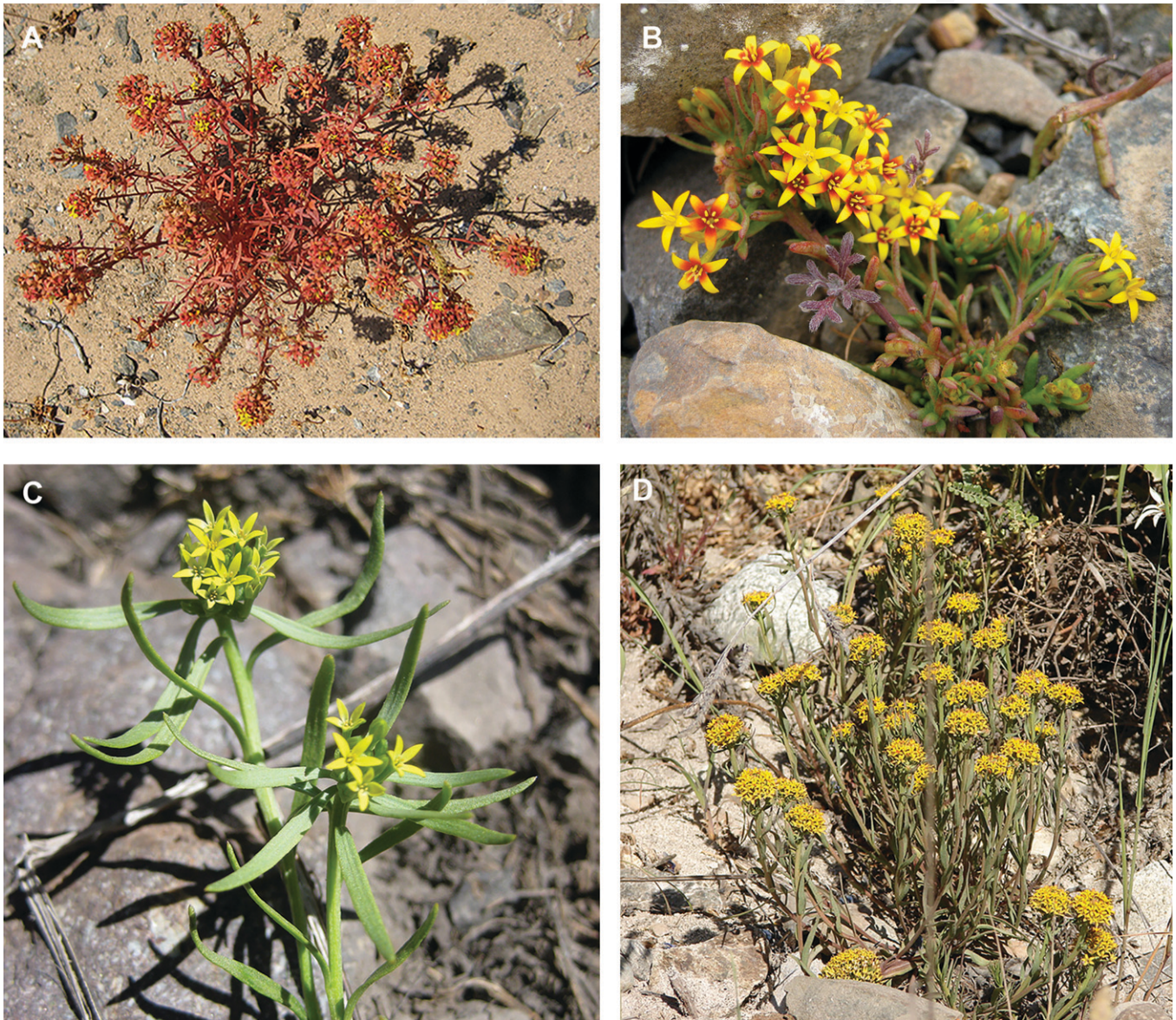


FIG. 1. Morphological variation in plants of *Quinchamalium*. A. Bariloche, Argentina. B. Paposo, Chile. C. Portillo, Chile. D. Huasco, Chile. Photographs by C. Calviño and R. Vidal Russell.

the relative importance of different factors that promote phenotypic differentiation (Ezcurra et al. 1997; Mascó et al. 2004; Chalcoff et al. 2008). *Quinchamalium* offers an excellent opportunity to test if the polymorphism it shows is interrupted by morphological gaps that could represent species discontinuities, or if its diversity represents environmentally induced morphological variation within a single species. Additionally, as expected in plants with extensive areas of distribution, this study could provide an opportunity to observe evolution in progress and understand the origin of systematic diversity.

Therefore, the main objective of this study is to clarify whether *Quinchamalium* is composed of more than one taxon or only a single polymorphic species. If the first scenario is true, we aim to find diagnostic characters that could be used to diagnose species or subspecies. If the second scenario is true, we attempt to identify biologically relevant climatic or geographic factors that may be influencing morphological variation.

To accomplish these objectives and to provide support for a taxonomic revision of the group, the present study evaluates the morphological variation existing in the genus. As with species complexes in many genera of angiosperms, we addressed this problem using multivariate analyses.

MATERIALS AND METHODS

Taxon Sampling for Species Delimitation—Approximately 750 herbarium specimens from BAB, BCRU, CONC, CORD, LP, MO and SI were examined to identify useful morphological traits for species delimitation. Many of these specimens could not be included in a morphometric study because of their inappropriate phenological stage (e.g. young emerging inflorescence that had not yet expanded, late fruiting specimens, or missing key floral parts), so adequate complete specimens were selected from 117 localities distributed throughout the geographic range of *Quinchamalium*, i.e. from northern Peru (ca. 7.1° S) to southern Argentina (ca. 50.4° S), from western Chile (ca. 73.35° W) to eastern Argentina (ca. 69.57° W), and from sea level to 3,800 m a.s.l., including the Bolivian

TABLE 1. Summary of comparison of morphometric variables in two putative species of *Quinchamalium*. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

N°	Characters	<i>Q. chilense</i> (N = 80)	<i>Q. parviflorum</i> (N = 37)	χ^2
Vegetative characters				
1	Stem length (ST)	20.28 ± 0.8 (7.5–40)	10.59 ± 0.79 (2.5–28)	34.34***
2	Leaf length (LFL)	17.79 ± 0.52 (8.4–33)	17.22 ± 1.27 (7.6–47.4)	0.92
3	Leaf width (LFW)	1.08 ± 0.03 (0.37–1.88)	0.85 ± 0.03 (0.36–1.78)	13.86***
Floral characters				
4	Leaf length: leaf width ratio (leaf shape) (LFSH)	18.01 ± 0.7 (6–43)	22.78 ± 1.7 (8.49–65)	4.92*
5	Corolla length (CL)	11.41 ± 0.16 (9–18)	6.62 ± 0.13 (5–8)	76.35***
6	Corolla width (CW)	0.9 ± 0.01 (0.43–1.29)	0.73 ± 0.01 (0.57–1)	30.67***
7	Corolla lobe length (LOBL)	3.89 ± 0.06 (2.2–5.39)	2.54 ± 0.08 (1.66–4.3)	60.86***
8	Corolla lobe width (LOBW)	1.12 ± 0.01 (0.69–1.64)	0.76 ± 0.02 (0.5–1.36)	58.08***
9	Calyculus length (CAL)	2.8 ± 0.04 (1.76–3.9)	2.5 ± 0.05 (1.87–3.23)	17.46***
10	Calyculus teeth length (CTL)	1 ± 0.02 (0.2–2)	0.68 ± 0.02 (0.32–1.3)	40.20***
11	Total filament length (FIL)	7.54 ± 0.18 (3.8–11)	4.77 ± 0.13 (3–7)	59.19***
12	Free filament length (FRFIL)	0.52 ± 0.03 (0–1.75)	0.36 ± 0.03 (0.1–1)	1.02
13	Nectary length (NL)	0.54 ± 0.02 (0.18–1.14)	0.49 ± 0.02 (0.2–1)	2.01
14	Anther length (AL)	1.48 ± 0.03 (0.82–2.77)	0.82 ± 0.04 (0.18–1.54)	56.52***
15	Anther width (AW)	0.37 ± 0.007 (0.23–0.65)	0.26 ± 0.01 (0.14–0.44)	35.93***
16	Style length (SL)	8.62 ± 0.22 (3.8–17.5)	5.2 ± 0.20 (3–9.3)	43.83***
17	Corolla length: style length ratio (floral morph) (FM)	1.42 ± 0.04 (0.9–3.25)	1.32 ± 0.03 (0.75–1.81)	0.45

Puna. These specimens were chosen to be representative of the whole morphological and ecological variation found in herbarium material.

Traits Measurements—Using previous analyses on the genus, descriptions of the taxa found in the literature, and personal observations, a list of potentially useful distinguishing traits was created. In the past, most of the original species descriptions in *Quinchamalium* were based upon morphological characters such as the shape of the leaves (Miers 1880) and the size of the flowers (Presl 1849; Philippi 1857). More recently, other characters have been used to distinguish species such as size of the floral bracts (Dawson 1944), and length of the free filament (Navas 1976). Therefore, in total, we used 17 morphological and floral traits that were scored on specimens usually pressed during anthesis. Measurements are based on rehydrated herbarium material, using hot water and [T1] detergent. The 17 variables were (Table 1; abbreviation given between parentheses): stem length (ST), leaf length (LFL), leaf width (LFW), leaf length: leaf width ratio as an estimator of leaf shape (LFSH), corolla length (CL), corolla width (CW), corolla lobe length (LOBL), corolla lobe width (LOBW), calyculus length (CAL), calyculus teeth length (CTL), total filament length (FIL), free filament length (FRFIL), nectary length (NL), anther length (AL), anther width (AW), style length (SL), and corolla length: style length ratio as an estimator of floral morph (FM). Because of considerable diversity in definitions of floral parts by different authors, [E2] floral characters measured are illustrated in Fig. 2.

Heterostylous species have two (distylous) or three (tristylous) floral morphs that differ reciprocally in the placement of their anthers and stigmas (Darwin 1877; Barrett 1992). Rivero et al. (1987) have reported distyly in the genus among populations of *Quinchamalium* of Volcán Casablanca in Chile. Therefore, to study the association between heterostyly and morphological, geographic and climatic variables, we calculated corolla length: style length ratio (floral morph) and implemented it as one of the 17 morphological quantitative traits used in the multivariate analyses (FM, Table 1).

Putative Species Delimitation—Because taxa limits are unclear and no key is available at present that includes the total number of species currently recognized, a preliminary hypothesis was tested. For this, two putative species were defined after a two-step procedure. First, every quantitative trait distribution was examined to identify potential variables without continuous variation and with gaps to differentiate among species. Only one variable (corolla tube length) was found with a bi-modal distribution. Wilcoxon tests were made to detect significant differences between these two putative species for the rest of the morphological variables. Second, in an exploratory principal components analysis (PCA) including all the continuous variables, the character that most contributed to the first axis was corolla length. Therefore, two putative species were proposed: plants with corolla lengths ≥ 9 mm in length (*Q. chilense* Molina), and those with corolla lengths < 9 mm in length (*Q. parviflorum* Phil.). To apply the oldest available species names within the genus to these two species, the types and protologues of all published names were consulted.

Morphometric Analyses—To detect homogeneous groups among specimens and extract the variables that best diagnose these groups, PCA was

performed. Variable contribution (loading values) to the PCA axes were interpreted as significant when ≥ 0.6 . Before conducting this analysis, all measurements were standardized. In addition, discriminant analysis (DA) of quantitative morphological variables was used to discriminate between the two pre-classified putative species. Prior to DA, the data matrix was modified in several ways. Because some quantitative characters were not normally distributed, they were \log_{10} transformed before the analyses. Traits that strongly correlated with other traits as determined by having a Pearson's correlation coefficient $r > 10.71$ were identified, and one of the traits of the correlating pair was excluded from the analysis (ST, LFSH, LOBL, CTL, FIL, FRFIL, AL, AW, SL, and FM). The trait corolla length (CL) was excluded because it was used previously in the identification of the two putative species. Finally, a subset of six continuous morphological variables was selected to conduct the DA (LFL, LFW, CW, LOBW, CAL, NL). In this study we considered a posteriori values of misclassification higher than 15% as indicator of low discriminant power. This threshold is commonly used in taxonomic studies (e.g. Lehnebach 2011). The PCA and the DA were carried out using the program JMP 9.0 (SAS Institute Inc. Cary, North Carolina).

Geographic and Climatic Variables—To determine the geographic range and climatic characteristics of the two putative species, collection data from herbarium specimens were examined, latitude and longitude

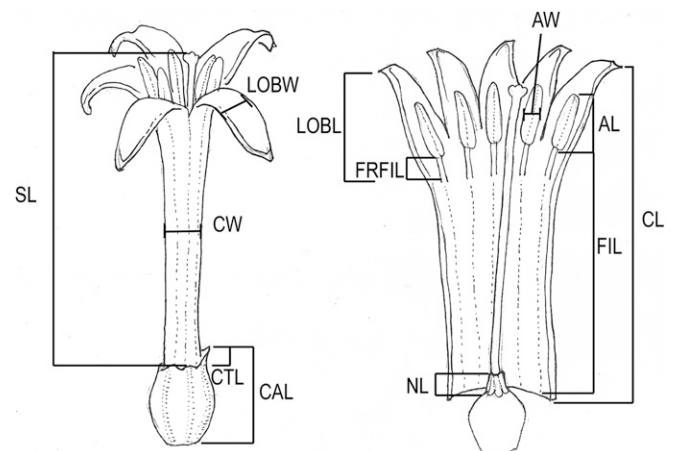


FIG. 2. Floral parts of *Quinchamalium chilense* included in morphological analyses. Left, closed flower. Right, open flower. CL: Corolla tube length. CW: Corolla tube width. LOBL: Corolla lobe length. LOBW: Corolla lobe width. CAL: Calyculus length. CTL: Calyculus teeth length. FIL: Filament length. FRFIL: Free filament length. NL: Nectary length. AL: Anther length. AW: Anther width. SL: Style length. Redrawn by C. Ezcurra from illustrations in Pilger (1935) and Rivero et al. (1987).

coordinates determined, and the distributions of each species plotted.

T3 19 bioclimatic variables (Table 3) derived from monthly temperature and rainfall values with a spatial resolution of 2.5 arc-minutes (5 km²) were downloaded from the WorldClim 1.4 database (<http://www.worldclim.org/current>; Hijmans et al. 2005). These environmental variables were extracted for each locality using QGIS 1.8.0-Lisboa (© 2002–2011 QGIS Development Team).

Association of Morphology with Climatic and Geographic Variation—We first measured the degree of association of the morphological variables with the climatic and geographic data, and among each type of data (Spearman correlation coefficients). Because of high correlations among climatic variables, we performed a PCA to generate two principal axes that were not correlated and accounted for the majority of variance of the climatic data. All variables were standardized (transformed into standard-deviation units) prior to performing the PCA. All individual morphological variables and the first two axes scores of the morphological PCA were regressed onto the first two axes scores of the climatic PCA. These analyses were performed to illustrate patterns of covariance between morphological traits and climate across the entire geographic range of *Quinchamalium*.

RESULTS

Putative Species Delimitation—None of the morphological variables explored with specimen frequency histograms showed gaps among groups of specimens, and corolla length was the only morphological character that showed a bi-modal **F3** distribution (Fig. 3). Two high frequency peaks resulted at two corolla length values (6 mm and 11 mm). The geographic distribution of the putative species, *Quinchamalium chilense* **F4** and *Q. parviflorum* is mapped in Fig. 4.

The mean values of corolla lengths were significantly different (Wilcoxon = 76.35, $p < 0.0001$), and separated two groups. Of the 17 morphological variables, 13 were also significantly different between putative species (Table 1).

Morphology: Principal Component Analysis—The first principal component (PC1) accounted 41.9% of the morphological variation, the second (PC2), 15.14%, and the third (PC3), only 8% of the total variance of the data. A scatterplot of the **F5** scores of principal components 1 and 2 (Fig. 5) partially separates two groups. Component 1 provides an incomplete separation between *Q. chilense* and *Q. parviflorum*. Individuals of *Q. parviflorum* (triangles) are dispersed entirely within the left side and have negative values for PC1 (-5–0). Individuals of *Q. chilense* (circles) are mainly positioned within the right side of the plane in a range of values of PC1 (-2–6).

Fourteen of the 17 variables employed in the PCA have relatively high loadings (absolute values >0.6) on at least

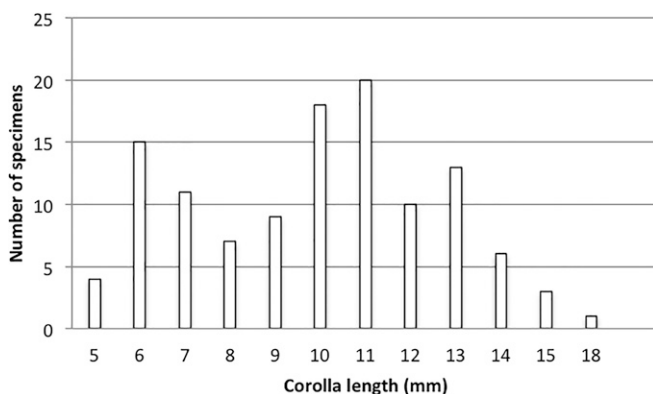


FIG. 3. Frequency histogram of corolla lengths of 117 plants of *Quinchamalium* in 1 mm intervals.

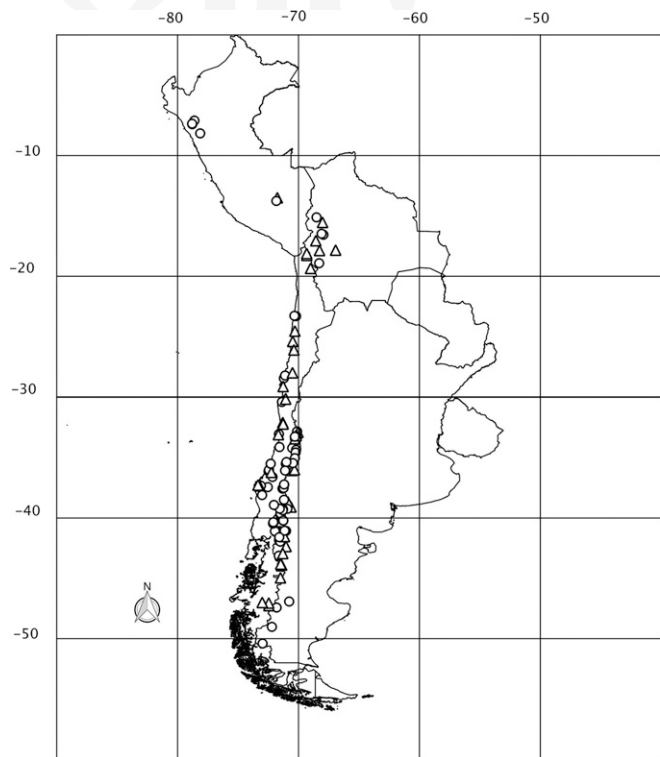


FIG. 4. Geographic distribution of 117 specimens studied of two putative species of *Quinchamalium* on southwestern South America (Perú, Bolivia, Chile, Argentina, Paraguay and Uruguay). Circles represent *Q. chilense* and triangles represent *Q. parviflorum*.

one of the first two components (Table 2). The first PC **T2** expressed mainly variation in plant and flower part size, e.g. stem length (ST), corolla length (CL), corolla lobe length (LOBL), anther length (AL), corolla lobe width (LOBW), corolla width (CW), and style length (SL; see Table 2 for all loadings), and tended to separate the individuals of the two putative species (Fig. 5). The second PC expressed mainly variation in leaf shape (LFSH), floral morph (FM), and free filament length (FRFIL; see Table 2 for all loadings). However, in this case, this component did not separate the two putative species (Fig. 5).

Morphology: Discriminant Analyses—In the discriminant analysis (DA), the function was significant (Function1: Wilk's Lambda = 0.511, $F = 17.2$, $p < 0.0001$). Overall, there is **some** overlap in the symbols of the two groups, but no overlap in the 95% confidence limits around group centroids (Fig. not shown). The DA of the two groups classified 83% of the specimens correctly into the groups to which they were a priori assigned to test the morpho-species hypothesis. Sixteen of the 80 individuals classified a priori as *Q. chilense* were misclassified, and four of the 38 individuals identified a priori as *Q. parviflorum* were misclassified.

Climate: Principal Component Analyses—Climatic data were well summarized by the first two principal components of PCA, which together accounted for 67% of the variation. Thirteen of the 19 variables employed in the PCA had relatively high loadings (absolute values >0.6) on one of the first two components (Table 3). The first PC of climate was dominated by temperature, with highest contributions of mean temperature of coldest quarter (BIO11), annual mean temperature (BIO1), mean temperature of wettest quarter

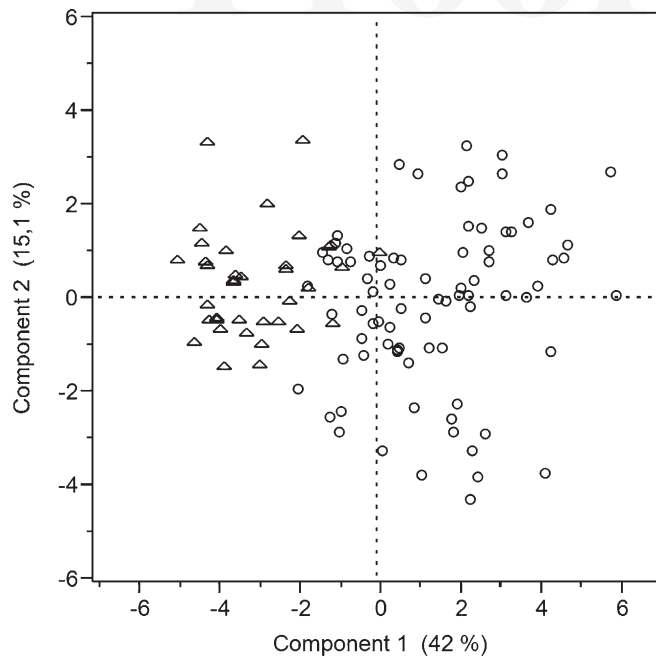


FIG. 5. Scatterplot of the first two principal components (PC1 and PC2) of the principal component analysis (PCA) of the 117 specimens of *Quinchamalium* studied based on 17 quantitative morphological variables. The variance explained associated with each PC is provided in parentheses. Symbols indicate the two putative species: *Q. chilense* circles, and *Q. parviflorum* triangles.

(BIO8), minimum temperature of coldest period (BIO6), and mean temperature of warmest period (BIO10; see Table 3 for all loadings). However, the PC2 of climate was dominated by precipitation factors: annual (BIO12), wettest quarter (BIO16), driest quarter (BIO17), wettest month (BIO13), coldest quarter (BIO19), and driest month (BIO14; see Table 3 for loadings).

Morphological Variation, Geography and Climate—All composite and some of the individual morphological variables were best predicted by climatic variables (Fig. 6A, B). The second principal axis of climate (dominated by precipi-

TABLE 2. Results of the principal component analysis (PCA) of the 17 morphological variables measured for 117 *Quinchamalium* specimens. The first two PCA axes accounted for 57% of the variance in the data. Values shown are the loadings of each variable on each of the first two PCA axes, with the highest contributing variables (>0.6) indicated in bold.

Variables	Description	Axis1	Axis2
ST	Stem length	0.651	0.178
LFL	Leaf length	0.288	0.575
LFSH	Leaf shape	-0.159	0.776
LFW	Leaf width	0.493	-0.437
CL	Corolla tube length	0.938	-0.078
CW	Corolla tube width	0.777	-0.110
LOBL	Corolla lobe length	0.885	0.130
LOBW	Corolla lobe width	0.810	-0.298
CAL	Calyculus length	0.583	0.066
CTL	Calyculus teeth length	0.691	-0.278
FIL	Filament length	0.749	-0.086
FRFIL	Free filament length	0.471	0.639
NL	Nectary length	0.244	0.226
AL	Anther length	0.858	0.026
AW	Anther width	0.700	-0.172
SL	Style length	0.734	0.447
FM	Floral morph	0.036	-0.713

TABLE 3. Results of the principal component analysis of the 19 climatic variables of 117 specimens of *Quinchamalium* studied. The first two principal axes accounted for 67% of the variance in the data. Values shown are the loadings of each variable on each of the first two axes.

Variables	Description	Axis 1	Axis 2
BIO1	Annual mean temperature	0.932	0.343
BIO2	Mean diurnal temperature range	-0.219	-0.365
BIO3	Isothermality	0.342	-0.307
BIO4	Temperature seasonality	-0.587	0.138
BIO5	Maximum temperature of warmest period	0.695	0.489
BIO6	Minimum temperature of coldest period	0.872	0.462
BIO7	Temperature annual range	-0.566	-0.139
BIO8	Mean temperature of wettest quarter	0.923	0.164
BIO9	Mean temperature of driest quarter	0.718	0.494
BIO10	Mean temperature of warmest quarter	0.834	0.438
BIO11	Mean temperature of coldest quarter	0.948	0.284
BIO12	Annual precipitation	-0.393	0.871
BIO13	Precipitation of wettest month	-0.357	0.792
BIO14	Precipitation of driest month	-0.454	0.783
BIO15	Precipitation seasonality	0.419	-0.442
BIO16	Precipitation of wettest quarter	-0.343	0.816
BIO17	Precipitation of driest quarter	-0.470	0.794
BIO18	Precipitation of warmest quarter	-0.012	0.358
BIO19	Precipitation of coldest quarter	-0.446	0.790

tation; Table 3) was the best predictor of the first morphological principal axis (dominated by corolla length; Fig. 6A). In contrast, this second principal axis of climate was not a good predictor of the second morphological principal component (results not shown). The first axis of climate (dominated by temperature; Table 3) was the best predictor of the second morphological principal component (dominated by leaf shape and floral morph; Fig. 6B). However, it was not a good predictor of the first morphological principal component (results not shown).

Floral morph was calculated as corolla length: style length ratio. Following Rivero et al. (1987), we interpreted flowers with small FM values as pin flowers (with relatively long styles), and flowers with high values of FM as thrum flowers (with relatively short styles). Therefore thrum flowers and wider leaves were more common in cooler sites while plants with pin flowers and narrower leaves were more predominant in warmer sites (Fig. 6B). In the same way, in general plants from wetter sites had larger flowers and were taller while plants of drier sites had smaller flowers and were smaller (Fig. 6A).

The degree of association between morphological variables and geographic data was dominated by the correlation of latitude with floral morph; in fact the most significant correlation among variables was between FM and latitude (Spearman's $\rho = -0.31$, $p = 0.0006$). Despite this, the two putative species with different floral sizes (*Q. chilense* and *Q. parviflorum*) are sympatric nearly the entire latitudinal and longitudinal distribution of *Quinchamalium* (Fig. 4).

DISCUSSION

This study presents the results of numerical analyses of morphological variation of vegetative and floral characters in *Quinchamalium*, a taxonomically understudied genus from the central and southern Andes. The existence of two putative species characterized by different corolla lengths was investigated. Although several other correlated traits showed significant differences between the two putative species, the

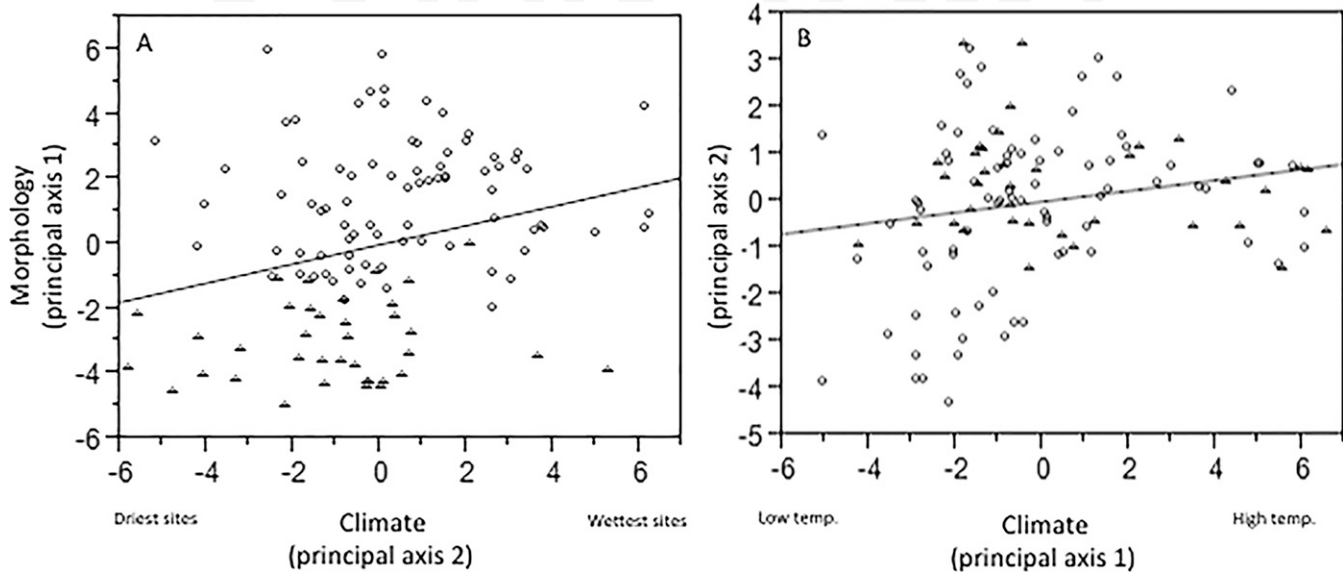


FIG. 6. Relationship between plant morphology and climate. A. The first morphological axis dominated by flower size and stem length (see Table 2 for all loadings) as a function of climate. Symbols are as in Fig. 4. The line is the best-fit linear regression. Slope = 0.29, $r^2 = 0.07$, $p = 0.0037$. In A, the predictor variable (x-axis) is the second principal axis of the PCA of climate, which is dominated by precipitation (see Table 3 for all loadings). B. The second morphological principal axis dominated by leaf shape and floral morph (see Table 2 for all loadings) as a function of climate. The line is the best-fit linear regression. Slope = 0.11, $r^2 = 0.037$, $p = 0.036$. In B, the predictor variable (x-axis) is the first principal axis of PCA of climate, which is dominated by temperature. (see Table 3 for all loadings).

multivariate analyses and the geographic distribution did not support them as different.

The results of PCA showed a partial separation of these two species, *Q. chilense* being generally characterized by larger flowers and taller plants, and *Q. parviflorum* by smaller flowers and shorter plants. However, multivariate analyses of quantitative characters were not useful for discriminating clearly among these two putative morpho-species, as evidenced by the overlap of their groups shown in the PCA scatterplot (Fig. 5) and the relatively low success rate (83% in the classification matrix) in the DA of the two putative species. In conclusion, and based on the practical criterion of morpho-species, our data do not support the existence of these two hypothesized species, inferring that only one single polymorphic species can be identified in the genus (*Q. chilense*; Lopez Laphitz et al. 2015).

Other important characters in relation to the morphological variability of the species were leaf shape and floral morph as shown by significant loadings in PC2, but these traits also did not support species separation. In the past, several authors (Presl 1849; Philippi 1857; Miers 1880; Dawson 1944; Navas 1976) already used leaf shape and floral size in taxonomic treatments of *Quinchamalium*, but they did not include numerical analyses. Therefore, our findings are the first ones that use these characters in quantitative multivariate analyses to determine if morpho-species exist. As additional evidence, the distribution map (Fig. 4) clearly shows that there are no geographic distinctions between the hypothesized morpho-species (*Q. chilense* and *Q. parviflorum*).

Considering the recognition of a single polymorphic and extended *Q. chilense*, geographic and climatic patterns associated with this morphological variation were studied. A geographical perspective is particularly important for species with broad distributions because they are likely to experience a wide variety of environmental conditions (e.g. Hodgins and Barrett 2008; Nicola et al. 2014). Significant associa-

tions between climate and morphology were observed for *Quinchamalium chilense*, e.g. sites with lower precipitations tended to have shorter plants with smaller flowers, and sites with higher temperatures had plants with relatively narrower leaves and pin flowers with relatively long styles. These types of differences suggest the effect of aridity gradients resulting from the combination of higher temperatures and reduced precipitations (e.g. Ezcurra et al. 1997; Quiroga et al. 2002). Larger plant and flower size associated with higher rainfall and more favorable climatic conditions for growth have been also observed in other plant groups (Hodgins and Barrett 2008).

By altering traits through phenotypic plasticity in response to changes in environmental conditions, plants are able to respond adaptively to a range of environments and thus use a wider range of habitats than would be possible if all traits were genetically fixed (Sultan 2000). But also, widespread species are likely to face a variety of conditions throughout their ranges that through natural selection can result in genotypic differentiation (Gaston 2003). Based on our results we cannot distinguish between these two alternatives for *Q. chilense*. The large morphological variation of this species resulting from heterogeneous environmental conditions could be the consequence of either genetic differences as inferred in the herb *Cerastium arvense* (Caryophyllaceae; Quiroga et al. 2002) or in the tree species *Nothofagus punilio* (Nothofagaceae; Premoli 2003; Premoli and Brewer 2007; Premoli et al. 2007), or phenotypic plasticity as described in *Ceanothus* (Rhamnaceae; Pugnaire et al. 2006) and *Psychotria* (Rubiaceae; Valladares et al. 2000). A common-garden experiment could differentiate between the two, but until now, cultivation of *Q. chilense* has not been successful; seeds failed to germinate and transplanted plants died after repeated attempts (Lopez Laphitz, pers. obs.).

Another important result of this study is the relation between floral morph and temperature. First, we observed

that geographic latitude was highly correlated with floral morph; the proportion of thrum floral morphs increasing to the south and pin morphs increasing to the north. At the same time, individuals with thrum flowers were not found to inhabit latitudes lower than 28° S (northern Chile: Huasco). Hence, northern regions (Peru, Bolivia and northern Chile) have exclusively pin flowers (long styles), while both morphs appear to be co-inhabitants of the southern portion of the distribution range of the species. It has been observed that similarly, many style-dimorphic *Narcissus* (Amaryllidaceae) display great variation in floral morph ratio, from dimorphic populations (pin: thrum or pin-biased) to pin-monomorphic populations in different geographic areas (Hodgins and Barrett 2008; Pérez-Barrales et al. 2014). This variation is frequently associated with shifts in pollinators, and the absence of one morph seems to be a derived condition (Hodgins and Barrett 2008; Pérez-Barrales et al. 2014). Secondly, as shown by the regression between the second axis of the morphological PCA and the first axis of the climatic PCA (Fig. 6A), at higher temperatures the corolla: style length ratio decreases, with a tendency to produce only pin flowers. Different patterns of flower morphology and floral-morph ratio have been found when plants are under selection by different functional groups of pollinators and in different climates (Chalcoff et al. 2008; Hodgins and Barrett 2008; Pérez-Barrales et al. 2014). Thus, temperature could be affecting floral morph indirectly by determining different pollinator assemblages in colder or warmer areas. Therefore, the different morph frequencies could be the result of specialization to a fraction of the environmental heterogeneity (abiotic or biotic) through selection processes, resulting in specialized taxa or ecotypes.

In conclusion, *Quinchamalium chilense*, a single, polymorphic, widespread species, shows a continuum of morphological variation because of either genotypic variation or phenotypic plasticity in response to environmental factors associated with latitude and climate. Considering the unified species concept (de Queiroz 2007), we cannot discard that *Q. chilense* may be showing different ecotypes or is in an early stage of species differentiation, with our results only presenting one view of this divergence. Future research combining molecular data could give support to the recognition of monophyletic groups with geographic, climatic and/or morphological coherence within this variable species.

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APPENDIX 1: Currently recognized *Quinchamalium* species after Brako and Zarucchi (1993), Zuloaga et al. (2009), and Jørgensen et al. (2014).

Nr.	Species	Author	Year of description	Distribution
1	<i>Q. andinum</i>	Philippi	1857	Chile (Región V, VIII, X)
2	<i>Q. bracteosum</i>	Philippi	1857	Chile (Región X)
3	<i>Q. brevistaminatum</i>	Pilger	1930	Perú (Dep. Lima)
4	<i>Q. carnosum</i>	Philippi	1857	Chile (Región II, III)
5	<i>Q. chilense</i>	Lamarck	1782	Argentina (Chubut, Jujuy, Mendoza, Neuquén, Río Negro, Santa Cruz) Chile (Región I, II, III, IV, V, VI, VII, VIII, IX, X, XI, RME) Bolivia (Deps. Cochabamba, La Paz, Oruro, Potosí)
6	<i>Q. elongatum</i>	Pilger	1930	Perú (Dep. Ancash)
7	<i>Q. excrescens</i>	Philippi	1857	Chile (Región II, III, IV, V)
8	<i>Q. fructulosum</i>	Steudel	1821–1824	Chile (Región VIII)
9	<i>Q. hopii</i>	Pilger	1930	Perú (Dep. Arequipa)
10	<i>Q. linarioides</i>	Philippi	1857	Chile (Región VII)
11	<i>Q. linifolium</i>	Meyen	1878	Chile (Región VII, VIII)
12	<i>Q. litorale</i>	Philippi	1864	Chile (Región V)
13	<i>Q. lomae</i>	Pilger	1930	Perú (Dep. Arequipa)
14	<i>Q. parviflorum</i>	Philippi	1857	Chile (Región IV, VII, RME)
15	<i>Q. pratense</i>	Philippi	1857	Chile (Región VIII)
16	<i>Q. procumbens</i>	Ruiz y Pavón	1799	Perú (Deps. Ancash, Apurímac, Arequipa, Cajamarca, Cuzco, Lima, La Libertad, Moquegua, Tacna).
17	<i>Q. purpureum</i>	Philippi	1857	Chile (Región VII)
18	<i>Q. tarapacanam</i>	Philippi	1891	Chile (Región I)
19	<i>Q. thesioides</i> var <i>thesioides</i>	Philippi	1860	Chile (Región II)
20	<i>Q. thesioides</i> var <i>flaccidum</i>	Philippi	1860	Chile (Región II)
21	<i>Q. stuebelii</i>	Hieronymus	1896	Perú (Dep. Puno) Bolivia (Dep. Oruro)